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Evaluation of a novel automated water analyzer for continuous monitoring of toxicity and chemical parameters in municipal water supply

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ABSTRACT

A novel tool, the DAMTA analyzer (Device for Analytical Monitoring and Toxicity Assessment), designed for fully automated toxicity measurements based on luminescent bacteria as well as for concomitant determination of chemical parameters, was developed and field-tested. The instrument is a robotic water analyzer equipped with a luminometer and a spectrophotometer, integrated on a thermostated reaction plate which contains a movable carousel with 80 cuvettes. Acute toxicity is measured on-line using a wild type *Photobacterium phosphoreum* strain with measurable bioluminescence and unaltered sensitivity to toxicants lasting up to ten days. The EC50 values of reference compounds tested were consistent with *A. fischeri* and *P. phosphoreum* international standards and comparable to previously published data. Concurrently, a laboratory trial demonstrated the feasibility of use of the analyzer for the determination of nutrients and metals in parallel to the toxicity measurements. In a prolonged test, the system was installed only in toxicity mode at the premises of the World Fair "Expo Milano-2015", a high security site to ensure the quality of the supplied drinking water. The monitoring program lasted for six months during which ca. 2400 toxicity tests were carried out; the results indicated a mean non-toxic outcome of $-5.5 \pm 6.2\%$. In order to warrant the system's robustness in detecting toxic substances, Zn was measured daily with highly reproducible inhibition results, $70.8 \pm 13.6\%$. These results assure that this novel toxicity monitor can be used as an early warning system for protection of drinking water sources from emergencies involving low probability/high impact contamination events in source water or treated water.

1. Introduction

Worldwide environmental laws regulating quality of water bodies are becoming more and more rigorous and demanding in both Western and Asian countries. In Europe, the Water Framework Directive (WFD, 2000) highlights the importance of a novel risk-based approach adopted to assess environmental status including chemical quality, ecological safety and morphological conditions. Thus, the integration of chemical and biological analysis in a single instrument can make this task viable and cost effective.

Toxicity tests are some of the most frequently used tools for the ecological and biochemical assessment of water quality. They are based on the detection of biological signals produced by microorganisms or higher organisms in response to changes in their environment, like the presence of toxic contaminants (Radix et al., 2000). Several instruments for bacteria-, algae-, invertebrate- and fish-based bioassays are now available in the market (review, see Kokkali and van Delft, 2014).

Risk assessment in aquatic environments based on toxicity assays often employs the determination of the inhibition effects produced by pollutants on the light emitted by luminescent microorganisms (Fernández-Piñas et al., 2014). Since the light emission phenomenon requires large quantities of bacterial energy, a difference in photon release is attributed to the effect of sample components which impair the bacterial metabolism. Consequently, the observed decrease of luminescence is regarded as proportional to the biotoxicity of the substances contained in the sample (Kaiser and Palabrica, 1991; Parvez et al., 2006).

Because of their sensitivity, ease and rapidity, the luminescent bacteria toxicity assays have been standardised for regulatory purposes with similar procedures in Europe, US and also in China (EN ISO 11348-3, 2009; ASTM D5660-96, 2009; GB/T 15441, 1995, respectively). The toxicity of the samples is usually expressed by the EC50 value, which is the amount of a pure substance or a sample at which 50% of luminescence inhibition is measured. In contrast to eukaryotic-based tests,

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the responses are quick since acute toxic effects are commonly calculated after 15 or 30 min of bacterial exposure (Kaiser, 1998). Environmental samples are usually analysed offline, with laboratory batch systems and portable devices, exploiting the light emitting properties of *Allivibrio fischeri*, *Photobacterium phosphoreum* and *Photobacterium leiognathi* bacteria (Bulich, 1979; Kuznetsov et al., 1999; Ulitzur et al., 2002; Ma et al., 2014; van de Merwe and Leusch, 2015).

On the other hand, threatened environments or specific situations relevant for public health, such as the water sources intended for human use, require continuous monitoring (van Wezel et al., 2010). Online luminescent bacteria toxicity monitoring via completely automated systems has been achieved by application of the flow-through technology (Kim and Gu, 2005; Pooley et al., 2004) and through batch systems incorporating two parallel lines for the analysis of reference and sample water (Lopez-Roldan et al., 2012). In the former configuration, the use of continuous flow technology poses the risk of cross-contamination and biofouling episodes, whereas, in the latter, dual-channel apparatus suffer from limited analytical frequency since sample analysis is intermittent and give only one test result in typically 15 – 30 min.

To overcome the problems associated with flow technology and significantly increase the analytical through-put, a novel robotic instrument for continuous monitoring of acute toxicity, DAMTA (device for analytical monitoring and toxicity assessment), was designed, realized and tested. The system was based on a direct reading detector incorporating 80 reaction cells in each of which a toxicity test can be run. The sentinel species was a wild type bioluminescent strain of *P. phosphoreum* isolated from the Tyrrhenian sea, characterized by exclusive performances in terms of luminescence, life-time and sensitivity. In a further embodiment of the system, the toxicological tests were combined with the possibility of parallel automated analysis of other relevant chemical parameters required for water quality assessment.

The main aim of this paper was to present the novel instrument DAMTA to scientists involved in toxicological and environmental analysis. The instrument has been tested in laboratory and field for the continuous monitoring of toxicity in combination with nutrient and metal analysis.

In particular, the analyzer was selected by the Authority responsible for the Integrated Water Service for the City of Milano to secure the safety of the water distribution network established for the World Fair "Expo Milano-2015", held in Milano, Italy, from May to October 2015. The system was installed in a high security site, possible target for terror attacks and was used as an early warning system as well as a technological guard to protect the drinking water distribution system.

2. Materials and methods

2.1. Instrument

DAMTA is a multi-parameter monitoring system housed in an industrial cabinet (82 (L) × 55 (W) × 184 (H) cm) and equipped with a LCD colour touch screen control pad. The storage, dispensation, incubation and measuring components were designed, built and assembled by Systea laboratories (Anagni, Italy). A 71 (L) × 40 (W) cm aluminum base plate accommodates the bacteria management device, the liquid handling system and a direct reading reaction tray (Fig. 1). The instrument was conceived to automatically perform in parallel toxicity tests (blue stream) and chemical analysis (red stream).

The groundwork of the acute toxicity assay is the bacteria management device that consists of a vial opener and a refrigerated compartment with three vials containing lyophilized bacteria. The analyzer was programmed to automatically open a new vial, reconstitute fresh bacteria, by adding up to 22 mL of rehydration buffer, discard bacteria in use when exhausted and repeat the same procedure on the next vial.

The liquid handling system is a robotic pipettor that can access samples, bacteria, buffer, blank water and reagent containers, for toxicity tests or chemical analysis. The mechanical arm has three degrees of freedom and is fitted with a stainless-steel needle that allows aspiration, transferring and dispensing of the fluids needed. All of them are added, in the suitable sequence and amount, directly inside 500 micro-litre cuvettes, duly chosen by the software among those available in the carousel. The sampler is equipped with a sensor for liquid level sensing, automated probe washing and sample dilution facilities. Aspiration volume ranges from 2 to 330 µL with 1 µL increment and ± 0.1 µL accuracy.

The thermostated direct reading movable reaction tray is capable to rotate in order to place any of the 80 reaction cells in front of the luminometer, the spectrophotometer or the washing station, according to the requested action. In toxicity tests, a rectangular optic fibre bundle carries out the luminescence signal to a remote and perfectly sealed photomultiplier module (PMT). The spectrophotometer used for chemical analysis is a direct reading, dual channel device supplied with a halogen lamp (6 V / 10 W) as light source and 9 narrow band automatically selectable interferential filters.

The analyzer is managed via dedicated software which provides overall control of both analytical operations and data acquisition functions by a GSM / GPRS device.

2.2. Chemicals and bacterial strain preparation

Reagents were purchased from Merck (Darmstadt, Germany) and they were of the highest purity grade available.

P. phosphoreum wt was isolated from the Tyrrhenian Sea and characterized by 16S ribosomal RNA gene sequencing by the Genomics Platform of Parco Tecnologico Padano (Lodi, Italy). A feature of this novel strain is that luminescence activity is expressed over a wide temperature range from 15 to 25 °C, but optimal sensitivity is observed at 25 °C. This may represent an advantage in case of monitoring under tropical conditions. Bacteria were grown and lyophilized at the Systea laboratories (Anagni, Italy), according to established protocols (Bulich, 1979).

The lyophiles of *P. phosphoreum* T3 mutation (PPT3), used as bioindicator organism in the Chinese standard for acute toxicity testing (GB/T 15441, 1995), were purchased from the Nanjing Institute of Soil Science, Chinese Academy of Science.

Stock solutions for toxicity tests were freshly prepared from > 98% pure substance (3,5-dichlorophenol) or certified 1000 mg L^{-1} standards (Cd (II), Pb (II), CN^-) or by dissolving metal sulfate (Cu (II), Zn (II)) or chloride (Hg (II)) salt in ultrapure water. As (III) and Cr (VI) stock solutions were made from sodium arsenite and potassium dichromate, respectively.

2.3. Automated toxicity tests

The following analytical steps were fully automated: (i) vial opening and bacterial rehydration; (ii) agitation of bacterial suspension for homogenization; (iii) aspiration and dispensing of bacteria, buffer, blank water, reference standards and samples; (iv) sample dilution and conditioning; (v) kinetic luminescence measurements of blanks, reference standards and samples; (vi) cleaning of dispensing needle and incubation cells; (vii) statistical analysis and calculation of EC50.

The analytical sequence was scheduled to run blanks, reference standards, undiluted and diluted samples, all in duplicate. Every analysis started and ended with a blank run such as to minimize the influence of inter-test bacterial variability. Samples or reference standards, water and osmotic buffer were mixed within the sampling arm before addition to the reaction cuvette.

As for the assay with *P. phosphoreum* wt, 0.2 g of freeze-dried bacteria were automatically rehydrated in 22 mL of proprietary rehydration buffer; 10^6 cells mL^{-1} of bacteria were added to blanks and samples and osmotically adjusted with proprietary NaCl-based buffer.

As for the assay with PPT3, 0.5 g of freeze-dried bacteria were